made by the current amendment is attached hereto as Exhibit A. A copy of all pending claims as amended herein is attached hereto as Exhibit B.

The claims have been amended to more particularly point out and distinctly claim that which Applicants regard as their invention. Claims 1-3, 5, 6 and 8-14 have been amended to correct minor formal errors. Claim 3 was further amended to correct an inadvertently omitted phrase. Support for this amendment can be found in the specification, for example, on page 9, lines 21-23. Claim 5 has been further amended to correct an obvious error. The word "negative" was left out following "reciprocal". Support for this amendment can be found throughout the specification, e.g. at page 10, line 15. Claims 6, 11 and 14 have been further amended to remove phrases containing the word "preferably". The subject matter of these phrases has been incorporated into new claims 15-17. Claim 13 was further amended to correct a missing trademark. No new matter has been added.

Applicants emphasize for the record that all amendments made in this prosecution history are not narrowing amendments made to overcome any "prior art" under 35 U.S.C. §§ 102 or 103 (unless expressly stated otherwise), and further that the amendments made herein are made solely for clarity, *i.e.* to more particularly point out and distinctly claim that which Applicants regard as their invention. Applicants expressly reserve the right to equivalents of all claim limitations to the full extent that they are currently available and that they may become available, *e.g.*, in the event that the *en banc* decision announced in *Festo Corp. v. Shoketsu Kinzoku Kogyo Co.*, 234 F.3d 558 (Fed. Cir. November 29, 2000) is modified or overturned by the Supreme Court of the United States (*cert. granted* June 18, 2001).

I. THE REJECTIONS UNDER 35 U.S.C. § 112

Claims 1-14 stand rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite.

Claims 6, 11 and 14 were deemed indefinite because the PTO alleged it is unclear whether the limitations following the phrase "preferably" were part of the claimed invention. Applicants have amended these claims, without narrowing their scope, to remove the phrase "preferably", thus making the rejection moot.

Claims 1-3 were deemed indefinite allegedly for insufficient antecedent basis for the limitations "the efficiency" and "the cycle number". Applicants respectfully traverse. "Inherent components of elements recited have antecedent basis in the recitation of the

components themselves. For example, the limitation 'the outer surface of said sphere' would not require an antecedent recitation that the sphere has an outer surface." See MPEP 2173.05(e). "The efficiency" and "the cycle number" are inherent components of the recited elements. An amplification of a target nucleic acid has an associated efficiency. Likewise, for each dilution of target nucleic acid and a set signal threshold value, there is a specific cycle number at which the signal threshold is exceeded. Thus, the efficiency and the cycle number have antecedent basis in the phrases "amplification of a target nucleic acid" and "for each dilution".

Claims 4 and 5 were deemed indefinite allegedly for insufficient antecedent basis for the limitations "the negative local 1st derivative" and "the reciprocal local 1st derivative", respectively. With respect to these limitations, Applicants respectfully traverse for the reasons set forth above regarding inherent properties. Once a non-linear continuously differentiable function has been determined in step e) of claims 2 and 3, respectively, then that function has but a single negative local 1st derivative. Thus, it is an inherent property of the mathematical function, and has antecedent basis in the function itself.

Claims 6 and 11-14 were deemed indefinite allegedly for insufficient antecedent basis for the limitation "the aid". Applicants have amended claims 6 and 11-14, without narrowing their scope, to remove this phrase, thus rendering the rejection moot.

Claims 7-10 were deemed indefinite allegedly for insufficient antecedent basis for the limitations "the absolute quantification", "the quantification" or "the relative quantification". With respect to this limitation, Applicants have amended these phrases, without narrowing their scope, to remove the word "the", thus making the rejection moot.

Claims 7-10 were also deemed indefinite allegedly for insufficient antecedent basis for the limitations "the original copy number", "the original ratio", or "the cycle number". With respect to this limitation, Applicants respectfully traverse for the reasons set forth above regarding inherent properties.

Claims 4 and 5 were rejected over the phrase "local 1st derivative". Applicants respectfully traverse. This phrase is part of "negative local first derivative" and "reciprocal negative local first derivative". Both these phrase are clearly defined in the specification at page 10, line 4 *et. seq.* "Applicant may be his or her own lexicographer as long as the meaning assigned to the term is not repugnant to the term's well known usage. In re Hill, 161 F.2d 367, 73 USPQ 482 (CCPA 1947)." See MPEP 2111.01.

It is respectfully submitted for the reasons given above that the claims are not indefinite. In view of the foregoing, Applicants submit that the above rejection of claims 1-14 under 35 U.S.C. § 112 has been obviated or overcome. Accordingly, the PTO is respectfully requested to reconsider and withdraw this rejection.

II. THE REJECTIONS UNDER 35 U.S.C. § 103

Claims 1-14 stand rejected under 35 U.S.C. § 103(a) over Wittwer et al. ("Wittwer") in view of Brown et al. ("Brown"). In response, Applicants respectfully disagree. The combination of Wittwer and Brown does not teach or suggest the invention as defined in the claims now pending for the reasons set forth below.

A. The Legal Standard

To reject claims in an application under 35 U.S.C. § 103, the Patent Office bears the initial burden of establishing a *prima facie* case of obviousness. *In re Bell*, 26 USPQ2d 1529, 1530 (Fed. Cir. 1993); MPEP § 2142. In the absence of establishing a proper prima facie case of obviousness, applicants who comply with the other statutory requirements are entitled to a patent. *In re Oetiker*, 24 USPQ2d. 1443, 1444 (Fed. Cir. 1992). In order to establish prima facie obviousness, three basic criteria must be met.

First, the prior art must provide one of ordinary skill in the art with a suggestion or motivation to modify or combine the teachings of the references relied upon by the PTO to arrive at the claimed invention. When an obviousness determination relies on one reference, there must be suggestion or motivation to modify the teaching of the reference in the manner suggested by the PTO. *In re Grabiak*, 226 USPQ 870 (Fed. Cir. 1985). Alternatively, when an obviousness determination relies on a combination of two or more references, there must be some suggestion or motivation to combine the references. *WMS Gaming Inc. v. International Game Technology*, 51 USPQ2d 1385, 1397 (Fed. Cir. 1999). The suggestion or motivation to combine the references generally arises in the references themselves, but may also be inferred from the nature of the problem or occasionally from the knowledge of those of ordinary skill in the art. *See id.* The mere fact that references could be modified or combined does not render the resultant modification or combination obvious unless the prior art also suggests the desirability of the modification or combination. *In re Mills*, 16 USPQ2d 1430 (Fed. Cir. 1990); MPEP § 2143.01.

Second, the prior art must provide one of ordinary skill in the art with a reasonable expectation of success. Thus, the skilled artisan, in light of the teachings of the prior art, must have a reasonable expectation that the modification or combination suggested by the PTO would succeed. *In re Dow*, 5 USPQ2d 1529, 1531-32 (Fed. Cir. 1988).

Third, the prior art, either alone or in combination, must teach or suggest each and every limitation of the rejected claims. *In re Gartside*, 53 USPQ2d 1769 (Fed. Cir. 2000). The teaching or suggestion to make the claimed invention, as well as the reasonable expectation of success, must come from the prior art, not Applicants' disclosure. *In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir. 1991). If any one of these criteria are not met, *prima facie* obviousness is not established, and Applicants are not required to show new or unanticipated results. *In re Grabiak*, 226 USPQ 870 (Fed. Cir. 1985).

B. Wittwer and Brown, Alone or in Combination, Do Not Teach Each and Every Element of Claims 1-8 and 15

Wittwer discloses methods for monitoring DNA amplification by fluorescence, either continuously or cycle-by-cycle. In one aspect of Wittwer, cycle-by-cycle monitoring is used to quantify PCR products. Brown discloses methods for quantifying nucleic acids using simultaneous amplification of a plurality of samples which may be serially diluted. Neither Wittwer or Brown, alone or in combination, teach or suggest the claimed invention.

The PTO asserts that "it would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to substitute and combine the preparation of a dilution of the target nucleic acid and fluorescent-labelled hybridization probe selected from TaqMan probes of Brown et al. in the method of sampling, amplifying and quantifying segment of nucleic acid of Wittwer et al."

First, Wittwer and Brown, alone or in combination, do not teach or suggest each and every element of Claims 1-8 or 15. Claim 1 is directed to a method of determining amplification efficiency of a target nucleic acid where, *inter alia,* a defined signal threshold is set and a cycle number where amplification results in a fluorescent signal that exceeds the threshold is determined for each dilution in a series. Importantly, for each dilution, amplification efficiency is determined as a function of the original amount of nucleic acid. Claims 2-8 and new Claim 15 depend from claim 1.

Wittwer does not teach or suggest determination of amplification efficiency as a function of the original amount of target nucleic acid. Wittwer, instead, teaches methods for

determination of the quantity of DNA in a sample. Furthermore, for determination of the quantity of DNA in a sample, Wittwer teaches interpolation methods rather than determination as a function of the original amount of the target nucleic acid. In Wittwer, these interpolation methods are applied to curves of fluorescence vs. cycle number from two samples having known copy numbers to determine the copy number of an unknown sample.

For instance, according to the example described in Wittwer, page 57, line 25 *et seq.*, curves of fluorescence vs. cycle number are generated from a series of initial known template copy numbers (See Fig. 18 of Wittwer). Based on a curve obtained with a sample of 50 ng of genomic DNA (but an unknown copy number), one of skill in the art, according to Wittwer, will recognize that there are at least 10^4 copies in the sample, but fewer than 10^5 copies (See Fig. 22 of Wittwer; basically the fluorescent signals obtained from the unknown sample fall between the signals obtained from 10^4 and 10^5 copies). Only these two surrounding copy numbers, i.e. a samples having 10^4 copies and a sample having 10^5 copies, are used to determine the copy number of the unknown sample by interpolation methods. Wittwer discusses two alternative interpolation methods to determine the copy number of an unknown DNA sample.

In one method, interpolation is performed along a single line, similar to conventional endpoint analysis. As shown in Wittwer, on page 58, lines 14-16, in order to perform interpolation along a horizontal line, a fluorescence threshold is established. As discussed above, only two curves from two surrounding samples having known copy numbers are used for interpolation. An arbitrary fluorescence threshold is set from which the cycle number needed to exceed that threshold is determined for each known sample and for the unknown sample. Presumably, the distance of the unknown sample from the two known samples determines the copy number of the unknown sample.

In contrast, claim 1 requires, *inter alia*, determining the cycle number at which a set fluorescence signal threshold is exceed for each dilution and determining amplification efficiency as a function of the original amount of target nucleic acid. In this method of interpolation, Wittwer does not teach or suggest setting fluorescence signal threshold for each dilution in a series. Nor does Wittwer even mention determining amplification efficiency in conjunction with the use of fluorescence thresholds.

Wittwer's only mention of amplification efficiency is in a second interpolation method. In this method, an entire curve of fluorescence vs. cycle number is interpolated (See

Wittwer, page 59, lines 3-20). Again, only the two curves corresponding to two samples having known copy numbers surrounding the unknown sample are used for interpolation. In one example, an exponential fit within the log-linear portion of each curve is attempted. From this, amplification efficiencies for the two known samples are determined. Then, these two amplification efficiencies are used to generate a single amplification efficiency for the unknown sample, from which the starting copy number of the unknown sample is determined. No fluorescence threshold is set using this method. Wittwer also discusses curve fitting (again for a plot of fluorescence vs. cycle number) using a sigmoidal curve that takes into account the lag phase, log-linear phase, and the plateau phase. Again, the need to set a fluorescence threshold is obviated, because all the relevant data points of the curve are used.

In contrast, claim 1 requires, *inter alia*, setting a defined signal threshold, determining the cycle number at which the signal threshold is exceeded for each dilution, and determining amplification efficiency as a function of the original amount of nucleic acid. Wittwer does not teach or suggest setting a defined signal threshold. For this method of interpolation, Wittwer teaches instead interpolating over the entire curve of fluorescence vs. cycle number. Nor does Wittwer teach or suggest determining amplification efficiency as a function of the original amount of target nucleic acid. What Wittwer teaches is the determination of the starting copy number of an unknown sample using the amplification efficiency of two samples having known copy numbers.

The PTO also refers to Figure 21 D of Wittwer as allegedly providing support for determining a non-linear continuously differentiable function of a logarithm of copy number of target nucleic acid used for the amplification as a function of the cycle number at which the signal threshold is exceeded and calculating the amplification efficiency from that function. Figure 21 D discloses a plot of coefficient of variances versus cycle number for different detection formats. It has no relevance in the context of determination of an amplification efficiency.

Combination with Brown fails to overcome the shortcomings of Wittwer. Brown does not teach determining amplification efficiencies or setting signal thresholds. Brown teaches instead only the use of dilutions in methods of detecting and quantifying nucleic acids.

Moreover, Wittwer actually teaches away from combination with Brown. Although Wittwer teaches a wide range of initial template copies (see, e.g., Fig. 18), Wittwer only uses two data curves surrounding an unknown sample to determine its copy number. There is simply no reason to interpolate more than two curves in a dilution series where only two curves that bracket an unknown sample are used. Thus, combination of Wittwer and Brown does not teach or suggest all of the claim limitations and Wittwer teaches away from a combination with Brown.

Furthermore, Wittwer does not teach or suggest each and every element of claims 2-6. Claims 2-6 recite, *inter alia*, determining a non-linear continuously differentiable function involving the logarithm of the copy number (or concentration) and cycle number. Wittwer does not teach determining a non-linear continuously differentiable function involving the logarithm of the copy number (or concentration) and cycle number. Wittwer only teaches curve fitting for a plot of fluorescence vs. cycle number. In addition, Wittwer does not teach each and every element of Claims 3 and 4. Wittwer does not teach determining negative and reciprocal local first derivatives of continuously differentiable functions as recited in Claims 3 and 4.

C. Wittwer and Brown, Alone or in Combination, Do Not Teach Each and Every Element of Claims 9-14 and 16-17

Second, Wittwer and Brown, alone or in combination, do not teach each and every element of Claims 9-14 and 16-17. Claim 9 and 10 recite methods of relative quantification of target nucleic acids where, *inter alia,* defined signal thresholds are set, a cycle number is determined for each dilution in each series where amplification exceeds the threshold, and continuously differentiable functions involving logarithm of the copy number and cycle number are determined. Wittwer, as discussed above, where a signal threshold is set, only utilizes two known surrounding copy numbers to determine the copy number of an unknown and does not teach or suggest determining continuously differentiable functions involving logarithm of the copy number and cycle number. Brown, as discussed above, teaches the use of dilution series and no more. Claims 11-14 and new Claims 16-17 depend from claim 10.

D. Claims 1-17 Satisfy 35 U.S.C. § 103

It is respectfully submitted for the reasons given above that the PTO has not established a *prima facie* case of obviousness against Claims 1-14. Applicants respectfully

request that the PTO withdraw the rejection under 35 U.S.C. § 103 of Claims 1-14. Applicants also submit that new Claims 15-17 meet the requirements for patentability under 35 U.S.C. § 103.

CONCLUSION

Applicants respectfully contend that all grounds for rejection have been overcome and/or obviated by the amendments and remarks set forth herein, and that Claims 1-17 are in condition for allowance. Accordingly, the PTO is respectfully solicited to allow claims. If any issues remain in connection herewith, the PTO is invited to telephone the undersigned to discuss same.

No fees are believed due in connection with this response. However, the Commissioner is authorized to charge all required fees, fees under 37 CFR § 1.17 and all required extension of time fees, or credit any overpayment, to Pennie & Edmonds U.S. Deposit Account No. 16-1150.

Respectfully submitted,

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Enclosures

EXHIBIT A
MARKED-UP VERSION OF AMENDED CLAIMS

1. (Amended) A method for determining the efficiency of an[the] amplification of a target nucleic acid comprising the steps of:

a) a dilution series of the target nucleic acid is prepared;

b) the target nucleic acid is amplified under defined reaction conditions and the amplification is measured in real-time;

c) a defined signal threshold value is set;

d) for each dilution the cycle number is determined at which the signal threshold value is exceeded; and

e) the amplification efficiency is determined as a function of the original amount of target nucleic acid.

2. (Amended) The method of claim 1, wherein the efficiency of an[the] amplification is determined by:

a) preparing a dilution series of the target nucleic acid;

b) amplifying the target nucleic acid under defined reaction and the amplification of the nucleic acid being measured in real-time;

c) setting a defined threshold value;

d) determining the cycle number for each dilution at which the signal threshold value is exceeded;

e) determining a non-linear continuously differentiable function of a logarithm of the copy number of target nucleic acid used for the amplification as a function of the cycle number at which the signal threshold value is exceeded; and

f) calculating the amplification efficiency E from the function determined in step e).

3. (Amended) The method of claim 1, wherein the efficiency of an[the] amplification is determined by:

a) preparing a dilution series of the target nucleic acid;

b) amplifying the target nucleic acid under defined reaction, the amplification of the nucleic acid being measured in real-time;

c) setting a defined threshold value;

d) determining the cycle number for each dilution at which the signal threshold value is exceeded;

e) determining a non-linear continuously differentiable function of the cycle number determined in step d) as a function of the logarithm of the copy number of target nucleic used in each case; and

f) calculating the amplification efficiency E from the function determined in step e).

5. (Amended) The method of claim 3, wherein the amplification efficiency E of a certain original amount of target nucleic acid is determined as the reciprocal negative local 1st derivative of the continuously differentiable function from step e).

6. (Amended) The method of claim 2, wherein the non-linear continuously differentiable function from step e) is determined with [the aid of] a polynomial fit[preferably of the 3rd, 4th, 5th, 6th or 7th degree].

8. (Amended) A method for [the] quantification of a target nucleic acid in a sample relative to a reference nucleic acid comprising the steps of:

a) determination of the amplification efficiencies of the target nucleic acid and of the reference nucleic acid under defined amplification conditions as claimed in claim 1;

b) amplification of the target nucleic acid contained in the sample as well as of the reference nucleic acid contained in the sample under the same defined amplification conditions;

c) measurement of the amplification of the target nucleic acid and of the reference nucleic acid in real time; and

d) calculation of the original ratio of target nucleic acid and reference nucleic acid in the sample by correction of the ratio derived from step c) with the aid of the amplification efficiencies determined in step a).

9. (Amended) A method for [the] relative quantification of a target nucleic acid relative to a reference nucleic acid and standardized with a calibrator sample comprising the steps of:

a) preparation of a common or two separate dilution series of target nucleic acid and reference nucleic acid;

b) amplification of the various dilutions of target nucleic acid and reference nucleic acid under defined reaction conditions, the amplification of the nucleic acid being measured in real-time;

c) setting defined signal threshold values for the target nucleic acid and reference nucleic acid;

d) determining the cycle number Cp to which the signal threshold values defined for the target nucleic acid and reference nucleic acid are exceeded in each dilution;

e) determining a continuously differentiable function of the Cp values determined in d) as a function of the logarithm of the amounts used of target nucleic acid and determining a continuously differentiable function of the determined Cp values as a function of the logarithm of the amounts used of reference nucleic acid;

f) determination of the Cp values of the target nucleic acid and reference nucleic acid in the sample to be analysed as well as in a calibrator sample;

g) assignment of the Cp values measured in step f) to a particular function values of the functions determined in step e);

h) calculating the quotients of the function values from g) of the target nucleic acid and reference nucleic acid for the sample to analysed as well as for the calibrator sample; and

i) determination of the ratio of the two quotients from h) as a measure for the original amount of target nucleic acid contained in the sample.

10. (Amended) A method for [the] relative quantification of a target nucleic acid relative to a reference nucleic acid and standardized with a calibrator sample comprising the steps of:

a) preparation of a common or two separate dilution series of target nucleic acid and reference nucleic acid;

b) amplification of the various dilutions of target nucleic acid and reference nucleic acid under defined reaction conditions, the amplification of the nucleic acid being measured in real-time;

c) setting defined signal threshold values for the target nucleic acid and reference nucleic acid;

d) determining the cycle number Cp at which the signal threshold values defined for the target nucleic acid and reference nucleic acid are exceeded in each dilution;
e) determining a continuously differentiable function of the logarithm of the amounts used of target nucleic acid as a function of the Cp values determined in d) and determining a continuously differentiable function of the logarithm of the amounts used of reference nucleic acid as a function of the determined Cp values;
f) determining the Cp values of the target nucleic acid and reference nucleic acid in the sample to be analysed as well as in a calibrator sample;
g) assignment of the Cp values measured in step f) to particular function values of the functions determined in step e);
h) calculating the quotients of the function values from g) of the target nucleic acid and reference nucleic acid for the sample to analysed as well as for the calibrator sample; and
i) determination of the ratio of the two quotients from h) as a measure for the original amount of target nucleic acid contained in the sample.

11. (Amended) The method of claim 10, wherein the continuously differentiable functions from step e) are determined with [the aid of] a polynomial fit[preferably of the 3^{rd}, 4^{th}, 5^{th}, 6^{th}, or 7^{th} degree].

12. (Amended) The method of claim 10, wherein the amplified nucleic acids are detected with [the aid of] at least one fluorescent-labelled hybridization probe.

13. (Amended) The method of claim 12, wherein the amplified nucleic acids are detected with [the aid] of FRET hybridization probes, molecular beacons, or TaqMan® probes.

14. (Amended) The method of claim 10, wherein the amplified nucleic acids are detected with [the aid of] a DNA-binding dye[, preferably with SybrGreen 1].